Journal of Applied Sciences Research, 3(10): 1218-1222, 2007

β-carbolines and Vitamin E Protect Against Nitric Oxide-induced Lipid Peroxidation in Rat Brain Homogenates

Amal A. Fyiad

Biochemistry Department. National Research Center, Dokki, Cairo, Egypt.

Abstract: Tryptoline and pinoline are two β-carbolines isolated from the nervous system of mammals. We have investigated the level of lipid peroxidation (LPO) in rat brain homogenates in the presence of nitric oxide (NO) which was released by the addition of sodium nitroprusside (SNP).We also examined the effect of other known antioxidants (vitamin E) on the NO-induced LPO. The concentration of malonaldehyde (MDA) plus 4-hydroxyalkenals (4- HAD) was used as an index of LPO.Incubation of (tryptoline, pinoline or vitamin E) with SNP (5 mM) increased MDA+4 HDA production in brain homogenates which were totally prevented by tryptoline, pinoline and vitamin E in a concentration dependent manner. Under the in vitro conditions of this experiment, vitamin E was more efficient than β-carbolines (pinoline and tryptoline) in limiting NO-induced LPO in rat brain homogenates.

Keywords: Tryptoline, Pinoline, Vitamin E, Nitric oxide, Lipid peroxidation, Brain homogenates

INTRODUCTION

Nitric oxide (NO) is an endogenously produced free radical which was initially characterized as endothelial derived relaxing factor [1].NO is now recognized also as a neurotransmitter in the central nervous system [2, 3]. The role of NO that is formed as a consequence of glutamate receptor activation has been extensively investigated. NO mediates biological actions ranging from vasodilatation, neurotransmission, inhibition of platelet adherence and aggregation, and macrophage – and neutrophil – mediated killing of pathogens[2]. High concentrations of NO are toxic and interact with superoxide (O2·-) to form peroxynitrite (ONOO-)[4]. ONOO- is a strong oxidant and, at physiological pH, it is protonated to form peroxynitrous acid (HOONO), a relatively long – lived oxidant. This decomposes spontaneously to form another potent oxidant with the reactivity of a hydroxyl-like radical[4,5], which could initiate lipidperoxidation[6].

Free radicals are molecules or atoms characterized by the presence of unpaired electron in its outer orbital. They are highly unstable, usually very reactive, and they have very short half-lives.The nervous system is highly sensitive to free radical injury because neurons have an elevated metabolic rate. Additionally, the brain contains high concentrations of iron and has relatively poorly developed antioxidant defense mechanisms[7,8].When free radicals react with the phospholipids of a biological membrane, they initiate a devastating chain reaction, identified as lipid peroxidation, that leads to less or suppression of numerous membrane-dependent cellular functions and even to the cell death [9]. Several molecules including malondaldehyde (MDA) and other aldehydes are produced as a consequence of lipid peroxidation[10].

The β-carbolines are an interesting class of psychoactive compounds [11] with a structural resemblance to the molecular frame work of the well known dopaminergic neurotoxin 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydro pyridine.

1, 2, 3, 4-tertrahydro-β-carboline (tryptoline) and 6-methoxy-1, 2, 3, 4-tetrahydro-β-carboline (pinoline) are two tricyclic compounds present in the brain with awide neuroanatomical distribution [12,13]. Although their physiological role is unclear, it has been reported that pharmacological concentrations of these compounds may inhibit the activity of the monoamine oxidase, they also reduce uptake of serotonin in the central nervous system[14,15]. Both β-carbolines show Sbenzodiazepines like anxyolytic effects in animals subjected to stress[15,16,17,18], and also increase brain serotonin levels[16].Tryptoline reduces the activity of complex I of the mitochondrial respiratory chain, the main source of free radicals *in vivo*[11]. Furthermore pinoline stabilize hepatic microsomal membrane against lipid Speroxidation induced by FeCl3, adenosine diphosphate (ADP) and nicotinamide Sadenine dinucleotide phosphate (NADPH)[19,20].

Recently it was proposed that β-carbolines may derive from indoles by condensation with aldehydes[21], which are generated during peroxidation of membrane lipids.

Corresponding Author: Amal A. Fyiad, Biochemistry Department. National Research Center, Dokki, Cairo, Egypt.

In the present study we tested the effect of tryptoline and pinoline in preventing lipid peroxidation induced in vitro by the addition of sodium nitroprusside (SNP), a spontaneous releaser of NO. We also compared the effect of vitamin E on NO-induced LPO in rat brain homogenates. The tissue concentration of malonaldehyde (MDA) plus 4-hydroxyalkenals (4-HDA) was used as an index of LPO[22].

MATERIALS AND METHODS

Chemicals: Sodium nitroprusside (SNP), tryptoline, pinoline and vitamin E were purchased from Sigma chemical Co., USA. The Bioxytech LPO-586 kit for lipid peroxidation was obtained also from Sigma. Other chemicals used were of the highest quality available.

Methods: Male Spargue- Dawley rats (body weight 200-230 g) were anesthetized and perfused through the heart with 0.9% NaCl(4°C). Immediately after perfusion, the brains were removed and kept frozen at -80°C until there homogenization with 20mM Tris-HCL buffer, pH 7.4. Aliquots of brain homogenates (3mg protein/ml) were incubated in a water bath at 37°C for 60 min with 5mM SNP in the presence or absence of either tryptoline, pinoline or vitamin E (0.001, 0.003, 0.01, 0.03, 0.1, 0.3mM). Lipid peroxidation was stopped by placing the homogenates into ice-cold water for 10 min at 4°C. LPO products in the supernatant were estimated using a Bioxytech LPO 586 kit.

Measurements of Malonaldehyde (MDA) and 4-hydroxyalkenal (4-HDA) levels and protein concentrations: MDA and 4-HDA levels were used as an index of LPO. These products were determined using the Bioxytech kit. In this assay, MDA and 4-HDA react with N-methyl-2-phenylindole, yielding a stable chromophore with a peak of maximum absorbance at 586nm.Results are expressed as the means ±SE of MDA+4-HDA n mol/mg protein.

Protein concentration was determined by the Bradford method using bovine serum albumin as a standard[23].

RESULTS AND DISCUSSIONS

As shown in (Fig.1), sodium nitoprusside significantly increased MDA+4-HDA levels in brain homogenates (P<0.001). SNP behaved as an excellent oxidant in terms of LPO, being more potent than H_2O_2[24]. The presence of tryptoline and pinoline reduced , in a concentration-dependent manner, the stimulatory effect on nitric oxide on lipid peroxidation.(Fig.1).

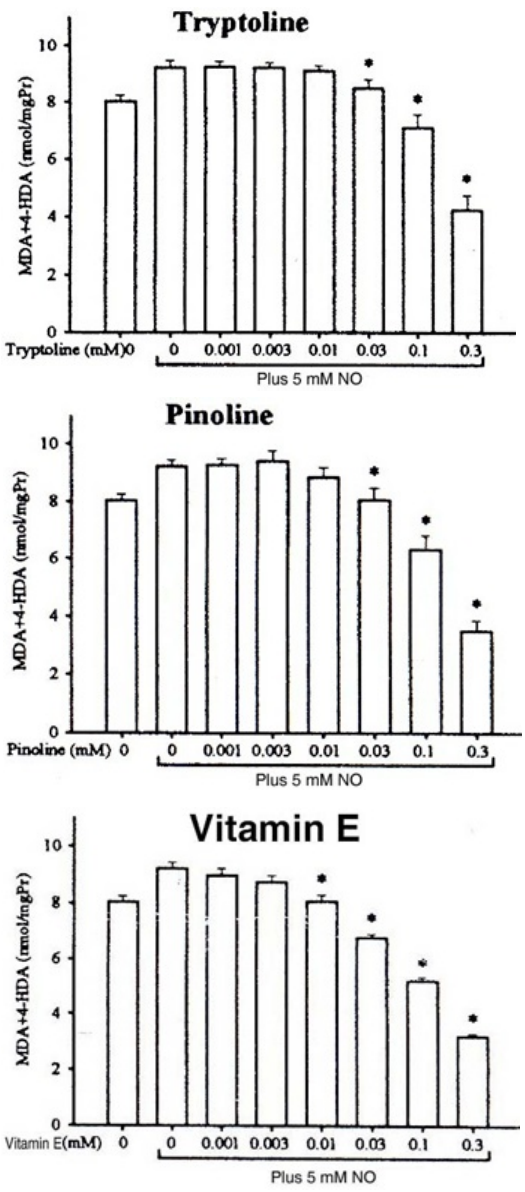

Fig. 1: The effect of several concentrations of tryptoline, pinoline, vitamin E on lipid peroxidation induced by nitric oxide (5 mM) in rat brain homogenates. The values represent the means ± SE obtained in three independent expirments.* p< 0.05 vs. nitric oxide.

Also, the effect of typtoline and pinoline on NO-induced LPO was compared with the antioxidant capability of vitamin E.

Vitamin E is a major lipid–soluble antioxidant which is concentrated in the hydrophobic interior of cell membranes [25,26]. Vitamin E is widely known to trap free radicals and to prevent peroxidative damge of membranes .

The present study shows that the concentration of tryptoline, pinoline and vitamin E required to inhibit the formation of MDA+4-HDA content by a 50% i.e (IC_{50}) were 0.98, 0.92 and 0.031 mM ($P<0.01$), respectively. Thus under the conditions of this in vitro study, vitamin E was more efficient than tryptoline and pinoline in preventing the NO-induced LPO in rat brain homogenates.

Also, Germaine et al., and Pinol et al.,[24,29] reported that vitamin E was more efficient than melatonin in limiting NO- induced lipid and protein peroxidation in rat brain homgenates.

The highest concentrations of tryptoline, pinoline and vitamin E used reduced MDA+4-HDA levels below control values (Fig.1).

Nitric oxide has proven to be an ubiquitous signal transduction molecule and a potent mediator of tissue injury because of its low molecular mass, volatility, lipophilicity, free radical nature, and diverse reactivities[30,31].

The present results show that NO is a potent LPO inducer, being even more efficient than H_2O_2. It has been recently shown that $OONO^-$ is a powerful oxidant that can initiate LPO in leucocytes[32]. $OONO^-$ is the product of the reaction between NO and $O_2^{\cdot-}$. Besides its degeneration to $OONO^-$, NO also undergoes numerous other reactions. NO is an iron ligand, and also reacts with iron-sulfur containing mitocondrial enzymes. Interestingly, NO has been observed to exhibit a protective role in pathological events associated with excess production and reactoin of partially reduced oxygen species. NO can intercept various lipid radicals forming adducts, which results in termination of chain propagation [30, 33]; furthermore, NO can scavenge Fenton-type oxidant and also, NO myoglobin and NO-Fe^{+2}- cysteine, by themselves, act as antioxidants[34]. Thus, the presence of $O_2^{\cdot-}$ diminishes the antioxidant nature of NO while enhancing the prooxidant function of NO.

Previously, it was reported that pinoline suppressed LPO in brain homogenates[29,35,36,37], a protective role that may be related to its ability to reduce oxidative stress-dependent membrane rigidity[20].

Pinoline also reduced nitric oxide- induced lipid peroxidation in retinal homogenates[38] and this carboline is found in the human retina[12]. It was also demonstrated that pinoline protects DNA against oxidative damage induced by chromium[39]. It is possible that pinoline scavenges hydroxyl radical generated from hydrogen peroxide in the presence of $CuSO_4$. This scavenging action may depend on both the indolic ring and the methoxy group, which are features common to pinoline[29,40].

Here it is shown, for the first time, that tryptoline also may reduce oxidative stress in brain homogenates. Tryptoline includes in its structure the indolic ring although it differs from pinoline in the absence of the methoxy group at position 5. It has been shown that methoxyindoles are better antioxidant than hydroxyindoles[41].

Tryptoline and pinoline are quickly distributed to all tissues and they easily cross the blood-brain barrier when they are peripherally administered to mice [42]. Another studies of the subcellular distribution of [^{3}H]-pinoline show that it is mainly localized in the cell nucleus[42], suggesting a potential role of this β-carboline in protecting DNA against free radical injury.

In conclusion CNS is the most sensitive tissue to oxidative damage due to free radicals. Until date, we knew that the mechanism responsible for initiation LPO was due to primarily the formation of $^{\cdot}OH$ or $NO^{\cdot}$. Now, we demonstrate that NO induces lipid peroxidation. Tryptoline, pinoline and vitamin E reduced in a dose-dependent manner this lipid peroxidation. Vitamin E was more potent than tryptoline or pinoline in preserving the lipid peroxidation induced by NO in the conditions used under this in vitro study.

REFERENCES

1. Fuchgott, R.F. and J.V. Zawadzki, 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine, Nature, 288: 373-376.
2. Moncada, S., R.M.J. Palmer and E.A. Higgs, 1991. Nitric oxide: Physiology, pathology and pharmacology, pharmacol. Rev, 43: 109-134.
3. Snyder, S.H. and D.S. Bredt, 1991. Nitric oxide as a neuronal messenger, Trend Pharmacol. Sci, 12: 125-128
4. Beckman, J.S., T.W. Beckman, J. Chen, P.A. Marshall and B.A. Free man, 1990. Apparent hydroxyl radical production by peroxinitrite: implications for endothelial injury from nitric oxide and superoxide, Proc. Natl. Acad. Sci. USA, 87: 1620-1624.
5. Yang, G., T.E.G. Candy, M. Boaro, H.F. Wilkin, P. Jones, N.B. Nazhat, R. Saadalla-Nazhat and D.R. Blake, 1992. Free radical yields from the homolysis of peroxynitrous acid, Arch. Biochem. Biophys, 288: 327-330.
6. Beckman, J.S., 1991. The double-edged role of nitric oxide in brain function and superoxide-mediated injury, J. Dev. Physiol, 15: 53-59.
7. Reiter, R.J., 1995. Oxidative processes and antioxidaative defense mechanisms in the aging brain, FASEB J, 9: 526-533.

8. Halliwel, B. and J.M.C. Gutteridge, 1999. Free radicals," reactive species" and toxicology In: Halliwell B, Gutteridge JMC (Eds) Free radicals in biology and medicine 3rd edition. Oxford university Press, New York, 544-616.
9. Kanner, J., J.B. German and J.E. Kinsella, 1987. Initiation of lipid peroxidation in biological systems, Crit. Rev. Food Sci, 25: 317-364.
10. Janero, D.R., 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury, Free Radic. Biol. Med, 9: 515-540.
11. Gerhard, B., F. Doris, B. Ralph, B. Michael, P. Karl , M.P. Eva, R. Heinz, J. Bernd, G. Christoph, W.C. Hans and W. Wolfgang, 2000. Bromal-drived Tetrahydro-β-carbolines as Neurotoxic Agents: Chemistry, Impairment of the Dopamine Metabolism, and Inhibitory Effects on Mitochondrial respiration, Bioorganic & Medicinal Chemistry, 8: 1467-1478.
12. Leino M., 1984. 6-Methoxy-tetrahydro-β-carboline and melatonin in the human retina, Exp. Eye Res, 38: 325-330.
13. Peura, P., J.V. Jhonson, R.A. Yost and K.F. Faull, Concentrations of tryptoline and methryptoline in rat brain, J. Neurochem, 25: 847-852.
14. Sparks, D.L. and N.S. Buckholtz, 1980. 6-methoxy-1,2,3,4-tetra-hydro-β-carboline: a specific monoamine oxidase- A inhibitor in CF-1 mouse brain, Neurosci. Lett., 20: 73-78.
15. Langer, S.Z., C.R. Lee, A. Segonzac, T. Tateishi, H. Esnaud, H. Schoemaker and B. Winblad,1984. Possible endocrine role of the pineal gland for 6-methoxytetrohydro-β-carboline a putative endogeous neuromodulator of the [^{3}H] imipramine recognition site, Eur. J. Pharm, 102: 379-380.
16. Mclsaac, W.M., D. Taylor, K.E. Walker and B.T. Ho, 1972. 6-methoxy-1, 2, 3, 4- tetrahydro-β-carboline-a serotonin elevator, J. Neurochem, 19: 1203-1206
17. Pähkla R, J. Harro and L. Rägo, 1996. Behavioural effects of pinoline in the rat forced swimming open field and elevated plus-maze tests, Pharmacol. Res, 34: 73-78.
18. Shekhar, A., J.N. Hingtgen and J.A. Dimicco, 1989. Anxiogenic effects of noreleagnine a water soluble beta-carboline in rats, Neuropharmacology, 28: 539-542.
19. Garcia, J.J., R.J. Reiter, J.M. Guerrero, G. Escames, B.P. Yu, C.S. Oh and Muñoz A. Hoyos, 1997. Melatonin prevents changes in microsomal membrane fluidity during induced lipid peroxidation, FEBS Lett, 408: 297-300.
20. Garcia, J.J., R.J. Reiter, J. Pie, G. Ortiz, J. Cabrera, R.M. Sainz and D. Acuna-Castroviejo, 1999. Role of pinoline and melatonin in stabilizing hepatic microsomal membranes against oxidative stress, J. Bioenerg. Biomembr, 31: 609-616.
21. Callaway, J.C., J. Gynther, A. Poso, J. Vepsäläninen and M.M. Airaksinen, 1994. The pictet-Spengler reaction and biogenic triptamines: formatting of tetrahydro-β-carbolines at physiological pH, J. heterocyclic Chem, 31: 431-435
22. Esterbauer, H. and K.H. Cheeseman, 1990. Determination of aldehyde lipid peroxidation products: malonaldehyde and 4-hydroxynonenal, Methods Enzymol, 186: 407-421.
23. Bradford, M.M., 1976. A rapid and sensitive methods for the quantification of microgram quantities of protein utilizing the principle of protein dye binding, Anal. Biochem, 72: 248-254.
24. Germaine, E., M.G. Juan, J.R. Russel, J.G. Joaquin, M.H. Antonio, G.O. Genaro and S.O. Chang, 1997. Melatonin and vitamin E Limit nitric oxide-induced Lipid peroxidation in rat brain homogenates, Neuroscience Letters, 230: 147-150.
25. Pieri, C., M. Marra, F. Moroni, R. Recchioni and F. Marcheselli, 1994. Melatonin: peroxyl radical scavenger more effective than vitamine E, Life. Sci, 55: 271-276.
26. Reiter, R.J., 1995. Oxidative processes and antioxidative defense mechanisms in the aging brain, FASEB J, 9: 526-533.
27. Heales, S.J.R., J.P. Bolanos, J.M. Land and J.B. Clark, 1994. Trolox protects mitochondrial complex IV from nitric oxide mediated damage in astrocytes, Brain Res, 668: 243-245.
28. Phoenix, J., R.H.T. Edwards and M.J. Jackson, 1989. Inhibition of Ca^{++} induced cylosolic enzyme efflux from skeletal muscle by vitamin E and related compounds, Biochem. J., 257: 207-213.
29. Pinol, R.G., Fuentes B.L., Millan P.S., R.R.J. Reyes, J.J. Garcia, Protective effect of melatonin and pinoline on nitric oxide-induced lipid and protein peroxidation in rat brain homogenates, Neurosci Lett, 405(1-2): 89-93
30. Rubbo, H., R. Radi, M. Trujillo, R. Telleri, B. Kalyanaraman, S. Barnes, M. Kirk and B.A. Freeman, 1994. Nitric oxide regulation of superoxide and peroxynitrite-dependent lipid peroxidation, J. Biol. Chem, 269: 26066-26075.
31. Wink, D.A., J.A. Cook, R. Paccelli, J. Liebmann, M.C. Krishna and J.B. Mitchell, 1995. Nitric oxide (NO) protects against cellular damage by reactive oxygen species, Toxicol. Lett, 82/83: 221-226.
32. Rodenas, J., M.T. Mitjavila and T. Carbonell, 1994. Simultaneous generation of nitric oxide and superoxide by inflammatory cells in rats, Free Radical Biol Med, 18: 869-875.

33. Hogg, N., V.M. Darley-Usmar, M.T. Wilson and S. Moncada, 1993. The oxidation of α-tocopherol in human low-density lipoprotein by the simultaneous generation of superoxide and nitric oxide, FEBS Lett, 326: 199-203.
34. Kanner, J., Harels and R. Granit, 1992. Nitric oxide, an inhibitor of lipid oxidation by lipooxygenase, cyclooxygenase and hemoglobin, Lipids, 24: 46-49.
35. Frederiksen, T.J.P., G. Pless, J.J. Garcia and R.J. Reiter, 1998. Pinoline and melatonin protect against H_2O_2-induced lipid peroxidation in rat brain homogenates, Neuroendocrin. Lett, 19: 117-123.
36. Pless, G., J.J.P. Frederiksen, J.J. Garcia and R.J. Reiter , 1999. Pharmacological aspects of N-acetyl-5-methoxytryptamine (melatonin) and 6-methoxy-1, 2, 3, 4-tetrahydro-β-carboline (pinoline) as antioxidants: reduction of oxidative damage in brain region homogenates, J. Pineal res, 26: 236-246.
37. Keynes, R.G., C.H. Griffiths, Hallc, J. Garthwaite, 2005. Nitric oxide consumption through lipid peroxidation in brain cell suspension and homogenates, Biochem. J, 387: (Pt 3) 685-94.
38. Siu, A.W., R.J. Reiter and C.H. To, 1999. Pineal indeleamines and vitamine E reduce nitric oxide. Induced lipid peroxidation in rat retinal homogenates, J. Pineal Res, 27: 122-128.
39. Qi, W., R.J. Reiter, D.X. Tan, L.C. Manchester, A.W. Siu and J.J. Garcia, 2000. Increased levels of oxidatively damaged DNA induced by chromium (III) and H_2O_2: protection by melatonin and rolated molecules, J. Pineal Res, 29: 54-61.
40. Pähkla, R., M. Zilmer, T. Kullisaar and Rägol, 1998. Comparison of the antioxidant activity of melatonin and in vitro, J. Pineal Res, 24: 96-101.
41. Tan, D.X., L.D. Chen, B. Poeggeler, 1993. Manchester LC and Reiter RJ, Melatonin: a potent endogenous hydroxyl radical scavenger, Endocr. J, 1: 57-60.
42. Pähkla, R., R. Masso, M. Zimler, L. Rägo and M.M. Airaksinen, 1996. Autoradiographic localization of [^{3}H]-pinoline binding sites in mouse tissues, Meth. Find. Exp. Clin. Pharmacol, 18: 359-366.